EFFECT OF BILOGICAL SOIL DISINFESTATIONS ON SOIL FUNGI

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Biological soil disinfestations (BSDs) using ethanol, wheat bran, rice bran, or molasses were developed as alternatives to chemical soil fumigation in Japan (Kobara et al. 2007, Shimura 2000). The methods are modified according to field conditions and used worldwide. BSDs effectively controlled *Fusarium oxysporum* f. sp. *lycopersici* (Momma et al. 2010), *Phytophthora capsici* (Rosskopf et al. 2010), black root rot of cucumber (Yokoyama et al. 2010), and Monosporascus root rot of water melon. Though these pathogens were strongly suppressed by BSDs, indigenous soil fungi were found to survive considerably after treatment (Momma 2010, Momma 2006). Here, a question arises as to the selectivity of BSDs.

We hypothesized that metal ions such as Fe^{2+} and Mn^{2+} and organic acids such as acetic acid and *n*-butyric acid generated in BSD-treated soil were important in suppressing *F. oxysporum* (Momma 2011, 2008). In this study, we compared the tolerances of fungi including plant pathogens and indigenous soil fungi to metal ions and organic acids.

Fusarium oxysporum f. sp. *lycopersici* and *Phomopsis sclerotioides* were grown on water agar (WA) and 1/10 potato dextrose agar (PDA). *Phytophthora capsici*, and *Monosporascus cannonballus* were grown on 1/10 PDA. After 7 days' incubation, agar disks were obtained using a cork borer. All pathogens were treated with 0.1% $FeSO_4$ for 12 days at 30 °C, transferred to PDA, and incubated for 12 days to confirm their viability. *P. sclerotioides*, *F. oxysporum*, and *M. cannonbollus* were suppressed irrespective of agar media used to obtain mycelial discs (Table 1). However, *P. capsici* survived the treatment. *P. capsici* prevails in aquatic environments where Fe^{2+} is readily generated. Thus, the fungi are likely to tolerate Fe^{2+}. Further studies are necessary using resting propagules.

Agar discs of the pathogens were soaked in 400 and 800 mg/l acetic acid or *n*-butyric acid solution, and were incubated at 30 °C for 24 hours. Viability of pathogens was determined the same way as above. *F. oxysporum* and *P. sclerotioides* were more sensitive to both organic acids when they were cultured on WA than on 1/10 PDA (Table 1). Both organic acids completely

suppressed *P. capsici* and *M. cannonballus* on 1/10 PDA.

A 2,000 g of fresh loamy sand (1,960 g dry weight) was packed into plastic container (ca. 1500 ml) equipped with screw cap lids with sealing rubber and then, 400 ml distilled water or 2% (v/v) ethanol solution was poured. Soil samples were incubated at 30 °C for 14 days. Neither *F. oxysporum* nor nematodes was detected from both soils treated with ethanol. In water treatments, *F. oxysporum* was detected at 496 cfu/g dry soil, and nematodes at 11 nematodes/20 g soil. These results confirmed the effectiveness of ethanol treatment. Soil was diluted to 10^{-3} (water treatment) or 10^{-2} (ethanol treatment) with distilled water and spread on Czapek's agar amended with chloramphenicol and rose bengal for enumeration and isolation of soil fungi.

Isolates were grouped according to their morphological characteristic and density of each group was calculated (Table 2). Fungi that had no similarity with any other isolates were lumped together and designated as "Others". All isolate was able to grow on WA. Agar plugs, which were obtained from 14-day-old culture, were treated with $FeSO_4$, acetic acid, and *n*-butyric acid and the viability was determined the same way as above. All representative isolate from 2% ethanol treatment exhibited tolerance to Fe^{2+} (Table 2). Except for Group IV isolate, all of them were also highly tolerant to both organic acids.

Our results suggested that plant pathogens might be generally more susceptible to Fe^{2+} and organic acids generated during BSDs than indigenous fungi. This would give a clue to answer the question; why were the pathogens suppressed whereas indigenous fungi were alive? Furthermore, this strengthens our hypothesis that Fe^{2+} and organic acids are involved in the mechanisms of biological soil disinfestation. In further study, the synergistic effect of Fe^{2+} and organic acids should be investigated.

Conclusions and Remarks

-Plant pathogens were more susceptible to Fe^{2+} and organic acids than indigenous soil fungi. This might be a cause of shifts in fungal community structure after BSDs treatment.

This study was supported by a grant "Research and development projects for application in promoting new policy of Agriculture Forestry and Fisheries (2019)" from the Japanese Ministry of Agriculture, Forestry and Fisheries.

Table 1 Effect of Fe^{2+} and organic acids on survival of plant pathogens.

pathogen	water	0.1% Fe^{2+}	acetic acid (mg/L)		*n*-butyric acid (mg/L)	
			400	800	400	800
Pregrown on WA						
Fusarium oxysporum	+ [1]	-	-	-	+	-
Phomopsis sclerotioides	+	-	±	-	-	-
Pregrown on 10% PDA						
F. oxysporum	+	-	+	+	+	+
P. sclerotioides	+	-	-	-	+	-
Phytophthora capsici	+	+	-	-	-	-
Monosporascus cannonballus	+	-	-	-	-	-

WA; water agar, PDA; potato dextrose agar

Agar plugs were treated with 0.1% $FeSO_4$ and organic acids and transferred to PDA to determine their viability.

[1] +: all three plugs viable, -: all three plugs dead, and ±: not all viable.

Table 2 Effect of Fe^{2+} and organic acids on survival of soil fungi.

Group	Soil treatment		0.1% Fe^{2+}	acetic acid (mg/L)		*n*-butyric acid (mg/L)	
	water	ethanol		400	800	400	800
I	2980 [2]	182	+	+	+	+	+
II	1324	-	-	+	-	+	-
III	497	-	-	+	-	+	+
IV	-	199	+	+	-	+	-
V	-	50	+	+	+	+	+
VI	-	99	+	+	+	+	+
VII	-	66	+	+	+	+	+
Others [1]	1159	50	NT	NT	NT	NT	NT

Indigenous soil fungi were isolated and grouped according to their colony morphology. Tolerance to Fe^{2+} and organic acids were determined as shown in Table 1.

[1] Total number of fungi that was dissimilar to any other isolates.

[2] CFU/g dry soil